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| <p>(21) International Application Number: PCT/GB90/00650</p> <p>(22) International Filing Date: 26 April 1990 (26.04.90)</p> <p>(30) Priority data: 8909916.2 29 April 1989 (29.04.89) GB</p> <p>(71) Applicant (<i>for all designated States except US</i>): DELTA BIOTECHNOLOGY LIMITED [GB/GB]; Castle Court, Castle Boulevard, Nottingham NG7 1FD (GB).</p> <p>(72) Inventor; and</p> <p>(73) Inventor/Applicant (<i>for US only</i>): BALLANCE, David, James [GB/GB]; 11 South Road, West Bridgford, Nottingham NG2 7AG (GB).</p> <p>(74) Agent: BASSETT, Richard; Eric Potter & Clarkson, St Mary's Court, St Mary's Gate, Nottingham NG1 1LE (GB).</p> | | <p>(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB, GB (European patent), HL, IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent), US.</p> <p>Published <i>With international search report.</i></p> | |
| <p>(54) Title: FUSION PROTEINS CONTAINING N-TERMINAL FRAGMENTS OF HUMAN SERUM ALBUMIN</p> <p>(57) Abstract</p> <p>A fusion polypeptide comprising, as at least part of the N-terminal portion thereof, an N-terminal portion of HSA or a variant thereof and, as at least part of the C-terminal portion thereof, another polypeptide except that, when the said N-terminal portion of HSA is the 1-n portion where n is 369 to 419 or a variant thereof, then the said polypeptide is one of various specified entities, including the 585 to 1578 portion of human fibronectin or a variant thereof. The HSA-like portion may have additional N-terminal residues, such as secretion leader sequences (signal sequences). The C-terminal portion is preferably the 585-1578 portion of human plasma fibronectin. The N-terminal and C-terminal portions may be cleavable to yield the isolated C-terminal portion, with the N-terminal portion having served to facilitate secretion from the host.</p> | | | |

One aspect of the present invention provides a fusion polypeptide comprising, as at least part of the N-terminal portion thereof, an N-terminal portion of HSA or a variant thereof and, as at least part of the C-terminal portion thereof, another polypeptide except that, when the said N-terminal portion of HSA is the 1-n portion where n is 369 to 419 or a variant thereof then the said polypeptide is (a) the 585 to 1578 portion of human fibronectin or a variant thereof, (b) the 1 to 368 portion of CD4 or a variant thereof, (c) platelet derived growth factor, or a variant thereof, (d) transforming growth factor, or a variant thereof, (e) the 1-261 portion of mature human plasma fibronectin or a variant thereof, (f) the 278-578 portion of mature human plasma fibronectin or a variant thereof, (g) the 1-272 portion of mature human von Willebrand's Factor or a variant thereof, or (h) alpha-1-antitrypsin or a variant thereof.

The N-terminal portion of HSA is preferably the said 1-n portion, the 1-177 portion (up to and including the cysteine), the 1-200 portion (up to but excluding the cysteine) or a portion intermediate 1-177 and 1-200.

The term "human serum albumin" (HSA) is intended to include (but not necessarily to be restricted to) known or yet-to-be-discovered polymorphic forms of HSA. For example, albumin Naskapi has Lys-372 in place of Glu-372 and pro-albumin Christchurch has an altered pro-sequence. The term "variants" is intended to include (but not necessarily to be restricted to) minor artificial variations in sequence (such as molecules lacking one or a few residues, having conservative substitutions or minor insertions of residues, or having minor variations of amino acid structure). Thus polypeptides which have 80%, preferably 85%, 90%, 95% or 99% homology with HSA are deemed to be "variants". It is also preferred for such variants to be physiologically equivalent to HSA; that is to say, variants preferably share at least one pharmacological utility with HSA. Furthermore, any putative variant which is to be used pharmacologically should be non-immunogenic in the animal (especially human) being treated.

Conservative substitutions are those where one or more amino acids are substituted for others having similar properties such that one skilled in the art of polypeptide chemistry would expect at least the secondary structure, and preferably the tertiary structure, of the polypeptide to be substantially unchanged. For example, typical such

substitutions include asparagine for glutamine, serine for asparagine and arginine for lysine. Variants may alternatively, or as well, lack up to ten (preferably only one or two) intermediate amino acid residues (ie not at the termini of the said N-terminal portion of HSA) in comparison with the corresponding portion of natural HSA; preferably any such omissions occur in the 100 to 369 portion of the molecule (relative to mature HSA itself) (if present). Similarly, up to ten, but preferably only one or two, amino acids may be added, again in the 100 to 369 portion for preference (if present). The term "physiologically functional equivalents" also encompasses larger molecules comprising the said sequence plus a further sequence at the N-terminal (for example, pro-HSA, pre-pro-HSA and met-HSA).

Clearly, the said "another polypeptide" in the fusion compounds of the invention cannot be the remaining portion of HSA, since otherwise the whole polypeptide would be HSA, which would not then be a "fusion polypeptide".

Even when the HSA-like portion is not the said 1-n portion of HSA, it is preferred for the non-HSA portion to be one of the said (a) to (h) entities.

The 1 to 368 portion of CD4 represents the first four disulphide-linked immunoglobulin-like domains of the human T lymphocyte CD4 protein, the gene for and amino acid sequence of which are disclosed in D. Smith et al (1987) Science 328, 1704-1707. It is used to combat HIV infections.

The sequence of human platelet-derived growth factor (PDGF) is described in Collins et al (1985) Nature 316, 748-750. Similarly, the sequence of transforming growth factors β (TGF- β) is described in Derzynck et al (1985) Nature 316, 701-705. These growth factors are useful for wound-healing.

A cDNA sequence for the 1-261 portion of Fn was disclosed in EP-A-207 751 (obtained from plasmid pFH6 with endonuclease PvuII). This portion binds fibrin and can be used to direct fused compounds to blood clots.

A cDNA sequence for the 278-578 portion of Fn, which contains a collagen-binding domain, was disclosed by R.J. Owens and F.E. Baralle in 1986 E.M.B.O.J. 5, 2825-2830. This portion will bind to platelets.

The 1-272 portion of von Willebrand's Factor binds and stabilises factor VIII. The sequence is given in Bonham et al. Nucl. Acids Res. 14, 7125-7127.

Variants of alpha-1-antitrypsin include those disclosed by Rosenburg et al (1984) Nature 312, 77-80. In particular, the present invention includes the Pittsburgh variant (Met³⁵⁸ is mutated to Arg) and the variant where Pro³⁵⁷ and Met³⁵⁸ are mutated to alanine and arginine respectively. These compounds are useful in the treatment of septic shock and lung disorders.

Variants of the non-HSA portion of the polypeptides of the invention include variations as discussed above in relation to the HSA portion, including those with conservative amino acid substitutions, and also homologues from other species.

The fusion polypeptides of the invention may have N-terminal amino acids which extend beyond the portion corresponding to the N-terminal portion of HSA. For example, if the HSA-like portion corresponds to an N-terminal portion of mature HSA, then pre-, pro-, or pre-pro sequences may be added thereto, for example the yeast alpha-factor leader sequence. The fused leader portions of WO 90/01063 may be used. The polypeptide which is

fused to the HSA portion may be a naturally-occurring polypeptide, a fragment thereof or a novel polypeptide, including a fusion polypeptide. For example, in Example 3 below, a fragment of fibronectin is fused to the HSA portion via a 4 amino acid linker.

It has been found that the amino terminal portion of the HSA molecule is so structured as to favour particularly efficient translocation and export of the fusion compounds of the invention in eukaryotic cells.

A second aspect of the invention provides a transformed host having a nucleotide sequence so arranged as to express a fusion polypeptide as described above. By "so arranged", we mean, for example, that the nucleotide sequence is in correct reading frame with an appropriate RNA polymerase binding site and translation start sequence and is under the control of a suitable promoter. The promoter may be homologous with or heterologous to the host. Downstream (3') regulatory sequences may be included if desired, as is known. The host is preferably yeast (for example Saccharomyces spp., e.g. S. cerevisiae; Kluyveromyces spp., e.g. K. lactis; Pichia spp.; or Schizosaccharomyces spp., e.g. S. pombe) but may be any

other suitable host such as E. coli, B. subtilis, Aspergillus spp., mammalian cells, plant cells or insect cells.

A third aspect of the invention provides a process for preparing a fusion polypeptide according to the first aspect of the invention by cultivation of a transformed host according to the second aspect of the invention, followed by separation of the fusion polypeptide in a useful form.

A fourth aspect of the invention provides therapeutic methods of treatment of the human or other animal body comprising administration of such a fusion polypeptide.

In the methods of the invention we are particularly concerned to improve the efficiency of secretion of useful therapeutic human proteins from yeast and have conceived the idea of fusing to amino-terminal portions of HSA those proteins which may ordinarily be only inefficiently secreted. One such protein is a potentially valuable wound-healing polypeptide representing amino acids 585 to 1578 of human fibronectin (referred to herein as Fn 585-1578). As we have described in a separate application (filed simultaneously herewith) this molecule contains cell spreading, chemotactic and chemokinetic activities

useful in healing wounds. The fusion polypeptides of the present invention wherein the C-terminal portion is Fn 585-1578 can be used for wound healing applications as biosynthesised, especially where the hybrid human protein will be topically applied. However, the portion representing amino acids 585 to 1578 of human fibronectin can if desired be recovered from the fusion protein by preceding the first amino acid of the fibronectin portion by amino acids comprising a factor X cleavage site. After isolation of the fusion protein from culture supernatant, the desired molecule is released by factor X cleavage and purified by suitable chromatography (e.g. ion-exchange chromatography). Other sites providing for enzymatic or chemical cleavage can be provided, either by appropriate juxtaposition of the N-terminal and C-terminal portions or by the insertion therebetween of an appropriate linker.

At least some of the fusion polypeptides of the invention, especially those including the said CD4 and vWF fragments, PDGF and α_1 AT, also have an increased half-life in the blood and therefore have advantages and therapeutic utilities themselves, namely the therapeutic utility of the non-HSA portion of the molecule. In the case of α_1 AT and others, the compound will normally be administered as

a one-off dose or only a few doses over a short period, rather than over a long period, and therefore the compounds are less likely to cause an immune response.

EXAMPLES : SUMMARY

Standard recombinant DNA procedures were as described by Maniatis et al (1982 and recent 2nd edition) unless otherwise stated. Construction and analysis of phage M13 recombinant clones was as described by Messing (1983) and Sanger et al (1977).

DNA sequences encoding portions of human serum albumin used in the construction of the following molecules are derived from the plasmids mHOB12 and pDBD2 (EP-A-322 094, Delta Biotechnology Ltd, relevant portions of which are reproduced below) or by synthesis of oligonucleotides equivalent to parts of this sequence. DNA sequences encoding portions of human fibronectin are derived from the plasmid pFHDELL, or by synthesis of oligonucleotides equivalent to parts of this sequence. Plasmid pFHDELL, which contains the complete human cDNA encoding plasma fibronectin, was obtained by ligation of DNA derived from plasmids pFH6, 16, 54, 154 and 1 (EP-A-207 751; Delta Biotechnology Ltd).

This DNA represents an mRNA variant which does not contain the 'ED' sequence and had an 89-amino acid variant of the III-CS region (R.J. Owens, A.R. Kornblith and F.E. Baralle (1986) Oxford Surveys on Eukaryotic Genes 3 141-160). The map of this vector is disclosed in Fig. 11 and the protein sequence of the mature polypeptide produced by expression of this cDNA is shown in Fig. 5.

Oligonucleotides were synthesised on an Applied Biosystems 380B oligonucleotide synthesiser according to the manufacturer's recommendations (Applied Biosystems, Warrington, Cheshire, UK).

An expression vector was constructed in which DNA encoding the HSA secretion signal and mature HSA up to and including the 387th amino acid, leucine, fused in frame to DNA encoding a segment of human fibronectin representing amino acids 585 to 1578 inclusive, was placed downstream of the hybrid promoter of EP-A-258 067 (Delta Biotechnology), which is a highly efficient galactose-inducible promoter functional in Saccharomyces cerevisiae. The codon for the 1578th amino acid of human fibronectin was directly followed by a stop codon (TAA) and then the S. cerevisiae phosphoglycerate kinase (PGK) gene transcription terminator. This vector was then introduced into S. cerevisiae by transformation, wherein it directed

the expression and secretion from the cells of a hybrid molecule representing the N-terminal 387 amino acids of HSA C-terminally fused to amino acids 585 to 1578 of human fibronectin.

In a second example a similar vector is constructed so as to enable secretion by S. cerevisiae of a hybrid molecule representing the N-terminal 195 amino acids of HSA C-terminally fused to amino acids 585 to 1578 of human fibronectin.

Aspects of the present invention will now be described by way of example and with reference to the accompanying drawings, in which:

Figure 1 (on two sheets) depicts the amino acid sequence currently thought to be the most representative of natural HSA, with (boxed) the alternative C-termini of HSA(1-n);

Figure 2 (on two sheets) depicts the DNA sequence coding for mature HSA, wherein the sequence included in Linker 3 is underlined;

Figure 3 illustrates, diagrammatically, the construction of mHOB16;

Figure 4 illustrates, diagrammatically, the construction of pHOB31;

Figure 5 (on 6 sheets) illustrates the mature protein sequence encoded by the Fn plasmid pFHDELL;

Figure 6 illustrates Linker 5, showing the eight constituent oligonucleotides;

Figure 7 shows schematically the construction of plasmid pDBDF2;

Figure 8 shows schematically the construction of plasmid pDBDF5;

Figure 9 shows schematically the construction of plasmid pDBDF9;

Figure 10 shows schematically the construction of plasmid DBDF12, using plasmid pFHDELL; and

Figure 11 shows a map of plasmid pFHDELL.

EXAMPLE 1 : HSA 1-387 FUSED TO Fn 585-1578

The following is an account of a preparation of plasmids comprising sequences encoding a portion of HSA, as is disclosed in EP-A-322 094.

The human serum albumin coding sequence used in the construction of the following molecules is derived from the plasmid M13mp19.7 (EP-A-201 239, Delta Biotechnology Ltd.) or by synthesis of oligonucleotides equivalent to parts of this sequence. Oligonucleotides were synthesised using phosphoramidite chemistry on an Applied Biosystems 380B oligonucleotide synthesizer according to the manufacturer's recommendations (AB Inc., Warrington, Cheshire, England).

An oligonucleotide was synthesised (Linker A) which represented a part of the known HSA coding sequence (Figure 2) from the PstI site (1235-1240, Figure 2) to the codon for valine 381 wherein that codon was changed from GTG to GTC:

Linker 1

| | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|
| | D | P | H | E | C | V |
| 5' | GAT | CCT | CAT | GAA | TGC | TAT |
| 3' ACGT | CTA | GGA | GTA | CTT | ACG | ATA |

1247

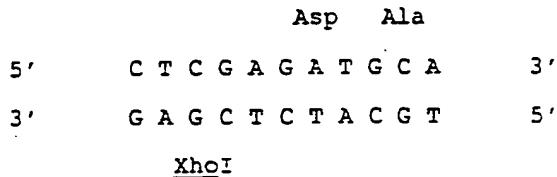
| | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|
| A | K | V | F | D | E | F | K |
| GCC | AAA | GTG | TTC | GAT | GAA | TTT | AAA |
| CGG | TTT | CAC | AAG | CTA | CTT | AAA | TTT |

1267

| | | |
|-----|-----|----|
| P | L | V |
| CTT | GTC | 3' |
| GGA | CAG | 5' |

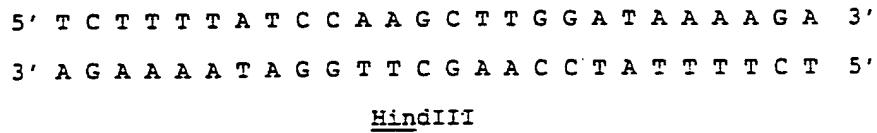
Linker 1 was ligated into the vector M13mp19 (Norrrander *et al*, 1983) which had been digested with PstI and HincII and the ligation mixture was used to transfect E.coli strain XL1-Blue (Stratagene Cloning Systems, San Diego, CA). Recombinant clones were identified by their failure to evolve a blue colour on medium containing the chromogenic indicator X-gal (5-bromo-4-chloro-3-indolyl- β -D-galactoside) in the present of IPTG (isopropylthio- β -galactoside). DNA sequence analysis of template DNA prepared from bacteriophage particles of recombinant clones identified a molecule with the required DNA sequence, designated mHOB12 (Figure 3).

M13mpl9.7 consists of the coding region of mature HSA in M13mpl9 (Norrrander *et al.*, 1983) such that the codon for the first amino acid of HSA, GAT, overlaps a unique XhoI site thus:



(EP-A-210 239). M13mpl9.7 was digested with XhoI and made flush-ended by S1-nuclease treatment and was then ligated with the following oligonucleotide (Linker 2):

Linker 2



The ligation mix was then used to transfect E.coli XL1-Blue and template DNA was prepared from several plaques and then analysed by DNA sequencing to identify a clone, pDBD1 (Figure 4), with the correct sequence.

A 1.1 kb HindIII to PstI fragment representing the 5' end of the HSA coding region and one half of the inserted oligonucleotide linker was isolated from pDBD1 by agarose gel electrophoresis. This fragment was then ligated with double stranded mHOB12 previously digested with HindIII and PstI and the ligation mix was then used to transfect E.coli XL1-Blue. Single stranded template DNA was prepared from mature bacteriophage particles of several plaques. The DNA was made double stranded in vitro by extension from annealed sequencing primer with the Klenow fragment of DNA polymerase I in the presence of deoxynucleoside triphosphates. Restriction enzyme analysis of this DNA permitted the identification of a clone with the correct configuration, mHOB15 (Figure 4).

The following oligonucleotide (Linker 3) represents from the codon for the 382nd amino acid of mature HSA (glutamate, GAA) to the codon for lysine 389 which is followed by a stop codon (TAA) and a HindIII site and then a BamHI cohesive end:

Linker 3

E E P Q N L I K J
5' GAA GAG CCT CAG AAT TTA ATC AAA TAA GCTTG 3'
3' CTT CTC GGA GTC TTA AAT TAG TTT ATT CGAACCTAG 5'

This was ligated into double stranded mHOB15, previously digested with HincII and BamHI. After ligation, the DNA was digested with HincII to destroy all non-recombinant molecules and then used to transfect E.coli XL1-Blue. Single stranded DNA was prepared from bacteriophage particles of a number of clones and subjected to DNA sequence analysis. One clone having the correct DNA sequence was designated mHOB16 (Figure 4).

A molecule in which the mature HSA coding region was fused to the HSA secretion signal was created by insertion of Linker 4 into BamHI and XhoI digested M13mp19.7 to form pDBD2 (Figure 4).

Linker 4

| M | K | W | V | S | F |
|----------|-----|-----|-----|-----|-----|
| 5' GATCC | ATG | AAG | TGG | GTA | AGC |
| G | TAC | TTC | ACC | CAT | TCG |
| <hr/> | | | | | |
| I | S | L | L | F | L |
| ATT | TCC | CTT | CTT | TTT | CTC |
| TAA | AGG | GAA | GAA | AAA | GAG |
| | | | | AAA | TCG |

| S | A | T | S | R | G | V | F |
|-----|-----|-----|-----|-----|-----|-----|-----|
| TCG | GCT | TAT | TCC | AGG | GGT | GTG | TTT |
| AGC | CGA | ATA | AGG | TCC | CCA | CAC | AAA |

R R
CG 3'
GCAGCT 5'

In this linker the codon for the fourth amino acid after the initial methionine, ACC for threonine in the HSA pre-pro leader sequence (Lawn *et al.*, 1981), has been changed to AGC for serine to create a HindIII site.

A sequence of synthetic DNA representing a part of the known HSA coding sequence (Lawn *et al.*, 1981) (amino acids 382 to 387, Fig. 2), fused to part of the known fibronectin coding sequence (Kornblhtt *et al.*, 1985) (amino acids 585 to 640, Fig. 2), was prepared by synthesising six oligonucleotides (Linker 5, Fig. 6). The oligonucleotides 2, 3, 4, 6, 7 and 8 were phosphorylated using T4 polynucleotide kinase and then the oligonucleotides were annealed under standard conditions in pairs, i.e. 1+8, 2+7, 3+6 and 4+5. The annealed oligonucleotides were then mixed together and ligated with mHOB12 which had previously been digested with the restriction enzymes HincII and EcoRI. The ligation

mixture was then used to transfect E.coli XL1-Blue (Stratagene Cloning Systems, San Diego, CA). Single stranded template DNA was then prepared from mature bacteriophage particles derived from several independent plaques and then was analysed by DNA sequencing. A clone in which a linker of the expected sequence had been correctly inserted into the vector was designated pDBDF1 (Fig. 7). This plasmid was then digested with PstI and EcoRI and the approx. 0.24kb fragment was purified and then ligated with the 1.29kb BamHI-PstI fragment of pDBD2 (Fig. 7) and BamHI + EcoRI digested pUC19 (Yanisch-Perron, et al., 1985) to form pDBDF2 (Fig. 7).

A plasmid containing a DNA sequence encoding full length human fibronectin, pFHDELL, was digested with EcoRI and XhoI and a 0.77kb EcoRI-XhoI fragment (Fig. 8) was isolated and then ligated with EcoRI and SalI digested M13 mp18 (Norrrander et al., 1983) to form pDBDF3 (Fig. 8).

The following oligonucleotide linker (Linker 6) was synthesised, representing from the PstI site at 4784-4791 of the fibronectin sequence of EP-A-207 751 to the codon for tyrosine 1578 (Fig. 5) which is followed by a stop codon (TAA), a HindIII site and then a BamHI cohesive end:

Linker 6

G P D Q T E M T I E G L
GGT CCA GAT CAA ACA GAA ATG ACT ATT GAA GGC TTG
A CGT CCA GGT CTA GTT TGT CTT TAC TGA TAA CTT CCG AAC

Q P T V E Y Stop
CAG CCC ACA GTG GAG TAT TAA GCCTTG
GTC GGG TGT CAC CTC ATA ATT CGAACCTAG

This linker was then ligated with PstI and HindIII digested pDBDF3 to form pDBDF4 (Fig. 8). The following DNA fragments were then ligated together with BclII digested pKV50 (EP-A-258 067) as shown in Fig. 8: 0.68kb EcoRI-BamHI fragment of pDBDF4, 1.5kb BamHI-StuI fragment of pDBDF2 and the 2.2kb StuI-EcoRI fragment of pFHDE11. The resultant plasmid pDBDF5 (Fig. 8) includes the promoter of EP-A-258 067 to direct the expression of the HSA secretion signal fused to DNA encoding amino acids 1-387 of mature HSA, in turn fused directly and in frame with DNA encoding amino acids 585-1578 of human fibronectin, after which translation would terminate at the stop codon TAA. This is then followed by the S.cerevisiae PGK gene transcription terminator. The

plasmid also contains sequences which permit selection and maintenance in Escherichia coli and S.cerevisiae (EP-A-258 067).

This plasmid was introduced into S.cerevisiae S150-2B (leu2-3 leu2-112 ura3-52 trp1-289 his3-1) by standard procedures (Beggs, 1978). Transformants were subsequently analysed and found to produce the HSA-fibronectin fusion protein.

EXAMPLE 2 : HSA 1-195 FUSED TO Fn 585-1578

In this second example the first domain of human serum albumin (amino acids 1-195) is fused to amino acids 585-1578 of human fibronectin.

The plasmid pDBD2 was digested with BamH I and BglII and the 0.79kb fragment was purified and then ligated with BamH I-digested M13mp19 to form pDBDF6 (Fig. 6). The following oligonucleotide:

5'-C C A A A G C T C G A G G A A C T T C G-3'

was used as a mutagenic primer to create a XhoI site in pDBDF6 by in vitro mutagenesis using a kit supplied by Amersham International PLC. This site was created by

changing base number 696 of HSA from a T to a G (Fig. 2). The plasmid thus formed was designated pDBDF7 (Fig. 9). The following linker was then synthesised to represent from this newly created XhoI site to the codon for lysine 195 of HSA (AAA) and then from the codon for isoleucine 585 of fibronectin to the ends of oligonucleotides 1 and 8 shown in Fig. 6.

Linker 7

D E L R D E G K A S S A K
TC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GCC AAA
A CTT GAA GCC CTA CTT CCC TTC CGA AGC AGA CGG TTT

I T E T P S Q P N S H
ATC ACT GAG ACT CCG AGT CAG C
TAG TGA CTC TGA GGC TCA GTC GGG TTG AGG GTG G

This linker was ligated with the annealed oligonucleotides shown in Fig. 3, i.e. 2+7, 3+6 and 4+5 together with XhoI and EcoRI digested pDBDF7 to form pDBDF8 (Fig. 9). Note that in order to recreate the original HSA DNA sequence, and hence amino acid sequence, insertion of linker 7 and the other oligonucleotides into pDBDF7 does not recreate the XhoI site.

The 0.83kb BamHI-StuI fragment of pDBDF8 was purified and then was ligated with the 0.68kb EcoRI-BamHI fragment of pDBDF2 and the 2.22kb StuI-EcoRI fragment of pFHDEL1 into BglIII-digested pKV50 to form pDBDF9 (Fig. 9). This plasmid is similar to pDBDF5 except that it specifies only residues 1-195 of HSA rather than 1-387 as in pDBDF5.

When introduced into S.cerevisiae S150-2B as above, the plasmid directed the expression and secretion of a hybrid molecule composed of residues 1-195 of HSA fused to residues 585-1578 of fibronectin.

EXAMPLE 3 : HSA 1-387 FUSED TO Fn 585-1578, AS CLEAVABLE MOLECULE

In order to facilitate production of large amounts of residues 585-1578 of fibronectin, a construct was made in which DNA encoding residues 1-387 of HSA was separated from DNA encoding residues 585-1578 of fibronectin by the sequence

I E G R
ATT GAA GGT AGA
TAA CTT CCA TCT

which specifies the cleavage recognition site for the blood clotting Factor X. Consequently the purified secreted product can be treated with Factor X and then the fibronectin part of the molecule can be separated from the HSA part.

To do this two oligonucleotides were synthesised and then annealed to form Linker 8.

Linker 8

| | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| E | E | P | Q | N | L | I | E | G |
| GAA | GAG | CCT | CAG | AAT | TTA | ATT | GAA | GGT |
| CTT | CTC | GGA | GTC | TTA | AAT | TAA | CTT | CCA |
| R | I | T | E | T | P | S | Q | P |
| AGA | ATC | ACT | GAG | ACT | CCG | AGT | CAG | C |
| TCT | TAG | TGA | CTC | TGA | GGC | TCA | GTC | GGG |
| N | S | H | | | | | | |
| TTG | AGG | GTG | G | | | | | |

This linker was then ligated with the annealed oligonucleotides shown in Fig. 6, i.e. 2+7, 3+6 and 4+5 into HincII and EcoRI digested mHOB12, to form pDEDF10

(Fig. 7). The plasmid was then digested with PstI and EcoRI and the roughly 0.24kb fragment was purified and then ligated with the 1.29kb BamHI-PstI fragment of pDBD2 and BamHI and EcoRI digested pUC19 to form pDBDF11 (Fig. 10).

The 1.5kb BamHI-StuI fragment of pDBDF11 was then ligated with the 0.68kb EcoRI-BamH1 fragment of pDBDF4 and the 2.22kb StuI-EcoRI fragment of pFHDEL1 into BalII-digested pKV50 to form pDBDF12 (Fig. 10). This plasmid was then introduced into S.cerevisiae S150-2B. The purified secreted fusion protein was treated with Factor X to liberate the fibronectin fragment representing residues 585-1578 of the native molecule.

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CLAIMS

1. A fusion polypeptide comprising, as at least part of the N-terminal portion thereof, an N-terminal portion of HSA or a variant thereof and, as at least part of the C-terminal portion thereof, another polypeptide except that, when the said N-terminal portion of HSA is the 1-n portion where n is 369 to 419 or a variant thereof then the said polypeptide is (a) the 585 to 1578 portion of human fibronectin or a variant thereof, (b) the 1 to 368 portion of CD4 or a variant thereof, (c) platelet derived growth factor or a variant thereof, (d) transforming growth factor β or a variant thereof, (e) the 1-261 portion of mature human plasma fibronectin or a variant thereof, (f) the 278-578 portion of mature human plasma fibronectin or a variant thereof, (g) the 1-272 portion of mature human von Willebrand's Factor or a variant thereof, or (h) alpha-1-antitrypsin or a variant thereof.

2. A fusion polypeptide according to Claim 1 additionally comprising at least one N-terminal amino acid extending beyond the portion corresponding to the N-terminal portion of HSA.
3. A fusion polypeptide according to Claim 1 or 2 wherein there is a cleavable region at the junction of the said N-terminal or C-terminal portions.
4. A fusion polypeptide according to any one of the preceding claims wherein the said C-terminal portion is the 585 to 1578 portion of human plasma fibronectin or a variant thereof.
5. A transformed or transfected host having a nucleotide sequence so arranged as to express a fusion polypeptide according to any one of the preceding claims.
6. A process for preparing a fusion polypeptide by cultivation of a host according to Claim 5, followed by separation of the fusion polypeptide in a useful form.
7. A fusion polypeptide according to any one of Claims 1 to 4 for use in therapy.

FIGURE 1

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FIGURE 1 CONT.

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FIGURE 2 DNA sequence coding for mature HSA

1C 20 30 40 50 60 70 80
 GATGCCA CACAAGAGT GAGGTTCTCA GCGGTTAAAGATTGGGAGAACAAAATTCAGGCGTGGGTTGATTCGCTT
 D A H K S E V A H R F K D L G E E N F K A I V L I A F
 90 100 110 120 130 140 150 160
 TGCTCA GTATCTTCAGCA GCTTCCATTAAGATCATGTAA AAAATTACTGAATGAACTAACTGAAATTGCAAAACATGTC
 A Q Y L Q Q C P F E D H V K L V N E V T E F A K T C
 170 180 190 200 210 220 230 240
 TTGCTGATGAGTCAGCTGAAATTTGACAAATCACTTCATAACCGTTTGAGACAAATTATGCACAGTGGCAACTCTT
 V A D E S A E N C D K S L H T L F G D K L C T V A T I
 250 260 270 280 290 300 310 320
 CGTGAAACCTATGGTGAATGGCTGACTGCTTCGAAACAGAAACCTGAGACAAATGAACTTGTGAACTTGTGAACTGAA
 R E T Y G E M A D C C A K Q E P E R N E S F L Q H R D
 330 340 350 360 370 380 390 400
 TGACACCCAAACCTCCCCGGATGGTGGAGACCAAGGGTTGATGTGATGACTGGCTTTTCATGACAAATGAAAGACAT
 D N P N L P R L V R P E V D V H C T A F H D N E E T
 410 420 430 440 450 460 470 480
 TTTTGAAAAAAATCTTATATGAAATGCCAGAAGACATGCTTACTTTATGCCCGGAACTCTTCTTGTGCTAAAGG
 P L K K Y L Y E I A R R H P Y F Y A P E I L F F A K R
 490 500 510 520 530 540 550 560
 TATAAAGCTCTTTACAGAATGTTGCCAGSTGCTGATAAAGCTGCCCTGCTGTTGCCAAAGCTGATGAACTTGGGA
 Y K A A F T E C C Q A A D K A A C I L P K L D E L R D
 570 580 590 600 610 620 630 640
 TGAAAGGGAAAGGCTTGTCTGCCAAAACAGAGACTCAAATGTGCCAGTCTCCAAAATTTGGAGAAAAGAGCTTCAAGCAT
 E G K A S S A K Q R L K C A S L Q K F G E R A F K A
 650 660 670 680 690 700 710 720
 GGGCAGTGGCTGGCTGAGGAGATTCGCAAAAGCTGAGTTTGCAAGAACTTCCAGTGTAGCAAGTCTTACCAA
 W A V A R I S Q R F P K A E F A E V S K L V C D I T K
 730 740 750 760 770 780 790 800
 GTCCACACCGAAATGCTGCCATGGAGATCTGCTGAAATGTGCTGATGACAGGGCGGACCTTGGCAAGTATACTGTGAAA
 V W T E C C H G D L L E C A D D R A D L A K Y E C E N
 810 820 830 840 850 860 870 880
 TCACGGATTGCACTCCAGTAAACTGAAGGAATGCTGTGAAAAACCTCTGTTGGAAAAATCCAACTGCAATTGGCGAAAGTGG
 Q D S I S S K L K E C C E K P I L E K S H C I A E V
 890 900 910 920 930 940 950 960
 AAAATGATGAGATGCTGCTGACTTGCCTTCATTAGCTGCTGATTTGTAAGACTAAAGGATTTGGAAAACATGCTG
 E N D E M P A D L P S L A A D F V E S K D V C K N Y A
 970 980 990 1000 1010 1020 1030 1040
 GAGGCGAAGGATGCTTCTGGCATTTTGATGAAATATGCCAAGAAGGGCTGCTGATTACTGCTGCTGCTGCTGCTGCT
 E A K D V F I G M F L Y E Y A R R H P D Y S V V I I

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FIGURE 2 Cont.

1050 1060 1070 1080 1090 1100 1110 1120
 GAGACTGCCAAAGACATATGAAAACCCTCTAGAGAACTGCCTGCGGCTCAGATCTCATGAATGCTGCCAAAGCT
 R L A K T Y E T T I E K C C A A D P H E S Y A K V

 1130 1140 1150 1160 1170 1180 1190 1200
 TCGATGAAATTAAACCTCTTGTGGAAAGACCTCTAGAATTAAATCAAACAAACCTGACCTTTTGACCGAGCTGGAGAC
 F D E F K P L V E E P Q N I K Q N C E I F S G L G E

 1210 1220 1230 1240 1250 1260 1270 1280
 TACAAAATTCCAGAAATGCCCTTAATTAGTCCTAACCAAGAAAGTACCCCAAGTGTCAACTCCACTCTGAGGGCTC
 Y K F Q N A L L V R Y T K K V P Q V S T ? T L V E V S

 1290 1300 1310 1320 1330 1340 1350 1360
 AAGAAAACCTAGGAAAAGTGGCAGCAAATGTTAAACATCCTGAAAGCAAAAGAAATGCCCTTGCAAGAAAGACTATCTAT
 R N L G K V G S K C C K H P E A K R M P C A E D Y I

 1370 1380 1390 1400 1410 1420 1430 1440
 CGGTGGCCCTGAAACCACTTATGTGTGTTGCATGAGAAAACGCCAGTAAGTCACAGACTCACAAAXTGTGACAGAGTCC
 S V V L N Q I C V L H E K T F V S D R V T K S C T E S

 1450 1460 1470 1480 1490 1500 1510 1520
 CTGGTGAAACAGCCGACCATGCTTTTCAGETCTGGAAAGTCATGAAACATAACGTTCCAAAGAGCTTAAGCTGAAACATT
 L V N R R P C F S A L E V D E T Y V P K E F N A E T F

 1530 1540 1550 1560 1570 1580 1590 1600
 CACCTTCCATGCAAGATATTCACACTTTCGAGAAGGAGAGCAATGAGAAAACACTGCACTTGAGCTGTGA
 T F H A D E C T L S E K E R Q I K K Q T A I V E I V

 1610 1620 1630 1640 1650 1660 1670 1680
 AACACAAAGCCAAAGGCAACAAAGAGCACTGAAAGCTTTATGGATGATTTGGAGCTTTTGAGAAAGTGTGCAAG
 K H K P K A T K E Q L K A V M D D F A A F V E X C C K

 1690 1700 1710 1720 1730 1740 1750 1760
 CCTGACGATAAGGAGACCTCTTGGGAGGAGGTTAAAACCTGCTGCAAGTCAGGTGCTTAAACA
 A D D K E T C F A E E G K K L V A A S Q A A L G L

 1770 1780
 TCTACATTTAAAGCATCTCAG

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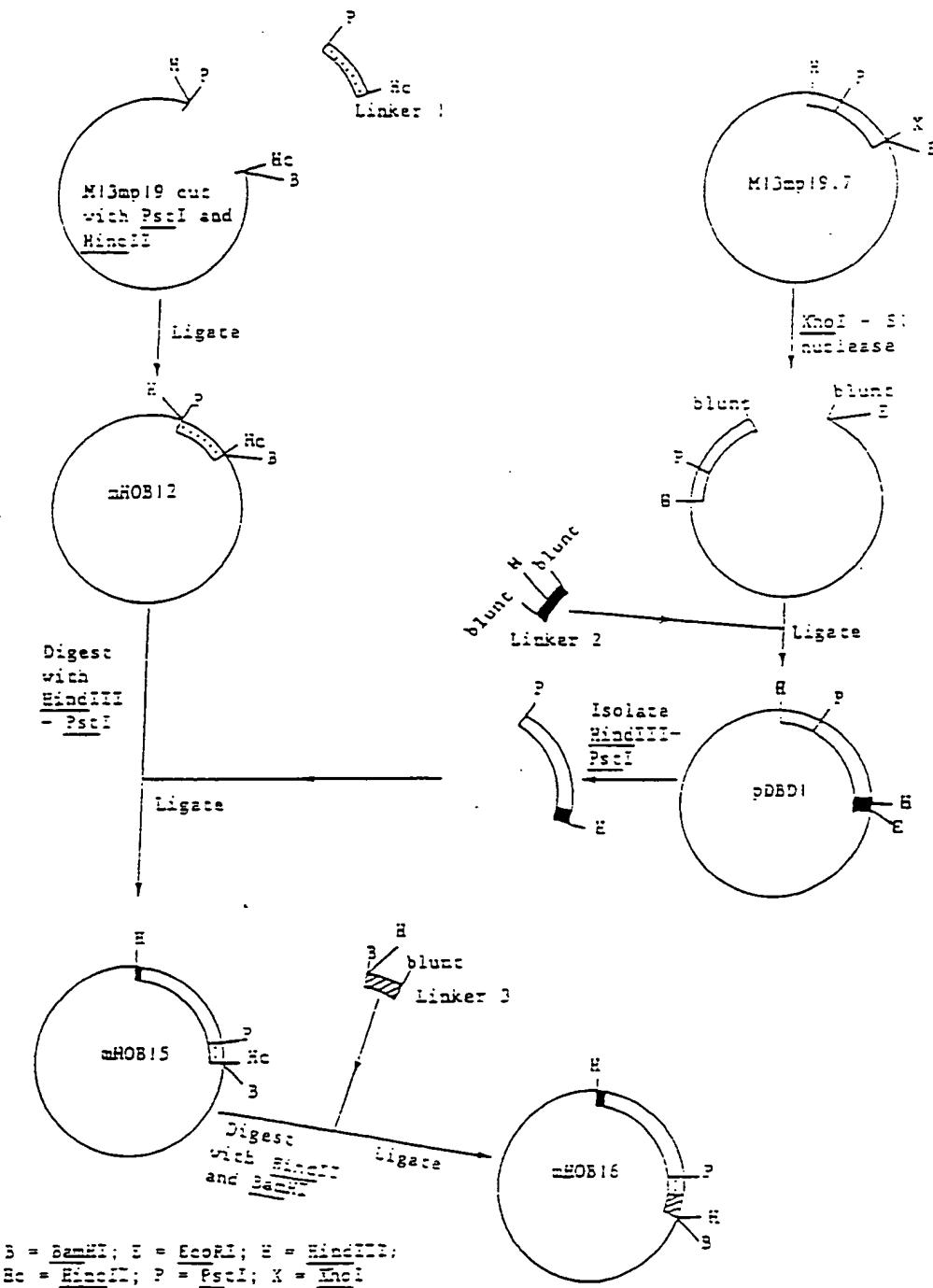
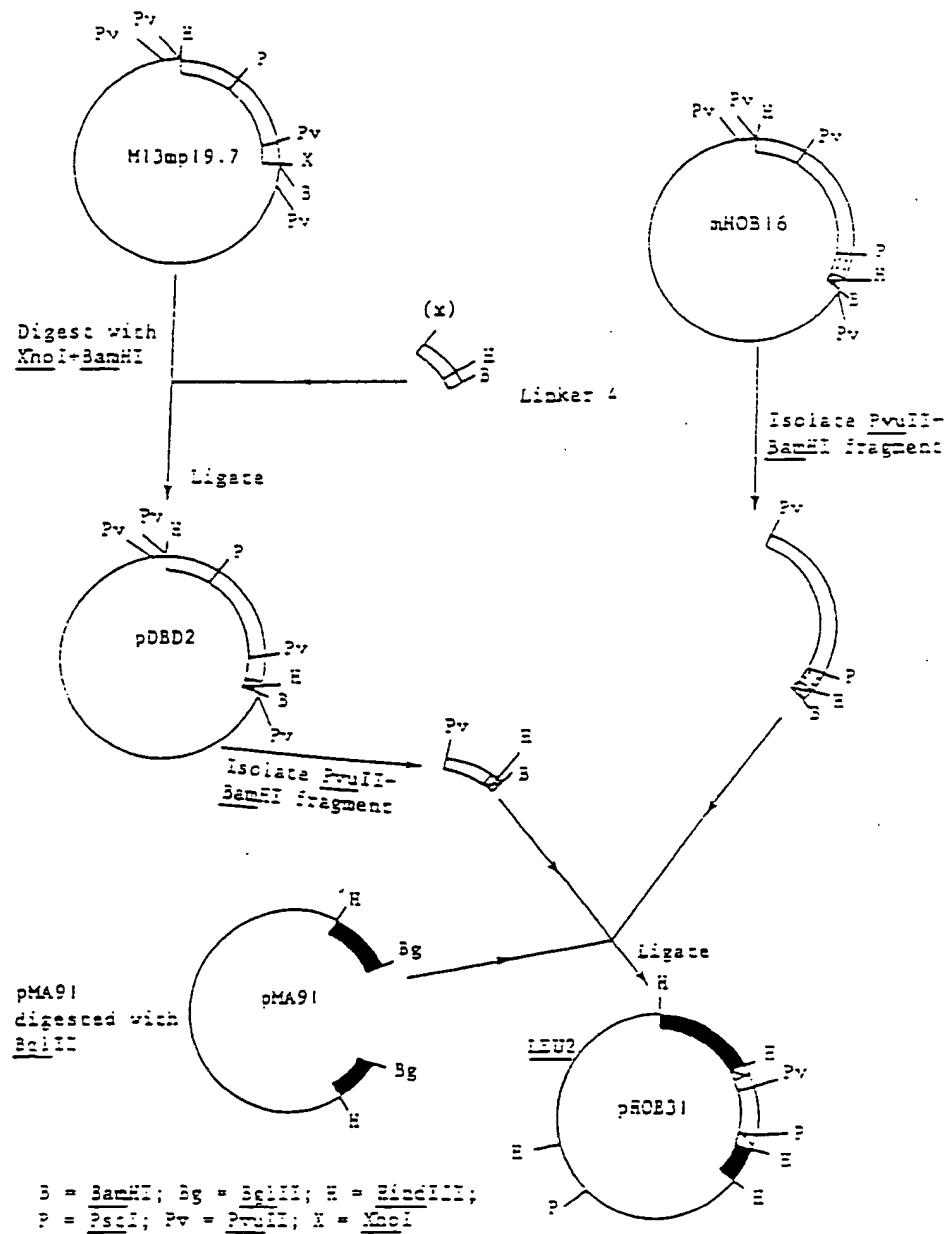
FIGURE 3 Construction of mHOB16**SUBSTITUTE SHEET**

FIGURE 4 Construction of pHOE31

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Gln Ala Gin Gin Met Val Gin Pro Gin Ser Pro Val Ala Val Ser Gin Ser Lys Pro Gly
 Cys Tyr Asp Asn Gly Lys His Tyr Gin 11e 30 Asn Gin Glu Trp Glu Arg Thr Tyr Leu Gly 40
 Asn Val Leu Val Cys Thr Cys Tyr Gly Gly Ser Arg Gly Phe Asn Cys Glu Ser Lys Pro 60
 Glu Ala Glu Glu Thr Cys Phe Asp Lys Tyr 70 Thr Gly Asn Thr Tyr Arg Val Gly Asp Thr 80
 Tyr Glu Arg Pro Lys Asp Ser Met 11e 90 Trp Asp Cys Thr Cys 11e Gly Ala Gly Arg Gly 100
 Arg 11e Ser Cys Thr 11e Ala Asn Arg Cys His Glu Gly Gly Gln Ser Tyr Lys 11e Gly 120
 Asp Thr Trp Arg Arg Pro His Glu Thr 130 Gly Gly Tyr Met Leu Glu Cys Val Cys Leu Gly 140
 Asn Gly Lys Gly Glu Trp Thr Cys Lys 150 Pro 11e Ala Glu Lys Cys Phe Asp His Ala Ala 160
 Gly Thr Ser Tyr Val Val Gly Glu Thr 170 Trp Glu Lys Pro Tyr Gin Gly Trp Met Met Val 180
 Asp Cys Thr Cys Leu Gly Glu Gly Ser 190 Arg 11e Thr Cys Thr Ser Arg Asn Arg Cys 200
 Asn Asp Gin Asp Thr Arg Thr Ser Tyr 210 Arg 11e Gly Asp Thr Trp Ser Lys Lys Asp Asn 220
 Arg Gly Asn Leu Leu Gin Cys 11e Cys 230 Thr Gly Asn Gly Arg Gly Glu Trp Lys Cys Glu 240
 Arg His Thr Ser Val Gin Thr Ser 250 Gly Ser Gly Pro Phe Thr Asp Val Arg Ala 260
 Ala Val Tyr Gin Pro Gin Pro His Pro 270 Gin Pro Pro Tyr Gly His Cys Val Thr Asp 280
 Ser Ely Val Tyr Ser Val Gly Met 290 Gin Trp Leu Lys Thr Gin Gly Asn Lys Gin Met 300
 Leu Cys Thr Cys Leu Gly Asn Gly Val 310 Ser Cys Gin Glu Thr Ala Val Thr Gin Thr 320
 Ely Gly Asn Ser Asn Gly Glu Pro Cys 330 Val Leu Pro Phe Thr Tyr Asn Gly Arg Thr Pha 340
 Tyr Ser Cys Thr Thr Cys Gin Arg Gin 350 Asp Gly His Leu Trp Cys Ser Thr Thr Ser Asn 360
 Tyr Glu Gin Asp Gin Lys Tyr Ser 370 Cys Thr Asp HIS Thr Val Leu Val Gin Thr Gin 380
 Ely Gly Asn Ser Asn Ely Ala Leu Cys 390 His Phe Pro Phe Leu Tyr Asn Asn His Asn Tyr 400
 Thr Asp Cys Thr Ser Glu Gly Arg Arg Asp Asn Met Lys Trp Cys Gly Thr Thr Gin Asn 420

Fig. 5A

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Tyr Asp Ala Asp Gln Lys Phe Gly Phe Gly Pro Met Ala His Glu Glu Ile Cys Thr
 Thr Asn Glu Gly Val Met Tyr Arg Ile Arg 430 Asp Gln Trp Asp Lys Gln His Asp Met 440
 450 His Met Met Arg Cys Thr Cys Val Gly Asn Gly Arg Gly Glu Trp Thr Cys Tyr Ala Tyr
 460 His Met Met Arg Cys Thr Cys Val Gly Asn Gly Arg Gly Glu Trp Thr Cys Tyr Ala Tyr
 470 Ser Gln Leu Arg Asp Gln Cys Ile Val Asp Asp Ile Thr Tyr Asn Val Asn Asp Thr 480
 490 His Lys Arg His Glu Glu Gly His Met Leu Asn Cys Thr Cys Phe Gly Gln Gly Arg 500
 510 Arg Trp Lys Cys Asp Pro Val Asp Gln Cys Gln Asp Ser Gln Thr Gly Thr Phe Tyr
 520 Ile Gly Asp Ser Trp Glu Lys Tyr Val His Gln Val Arg Tyr Gln Cys Tyr Cys Tyr 530
 540 Arg Gly Ile Gly Glu Trp His Cys Gln Pro Leu Gln Thr Tyr Pro Ser Ser Gly 550
 560 Val Ile Val Phe Ile Thr Glu Thr Pro Ser Gln Pro Asn Ser His Pro Ile Gln Trp Asn
 570 Ala Pro Gln Pro Ser His Ile Ser Lys Ile Leu Arg Trp Arg Pro Lys Asn Ser 580
 590 Gly Arg Trp Lys Glu Ala Thr Ile Pro Gln His Leu Asn Ser Tyr Thr Ile Lys Gly Leu
 600 Lys Pro Gly Val Val Tyr Glu Gly Gln Ile Ser Ile Gln Gln Tyr Gly His Gln Gln
 610 Val Thr Arg Phe Asp Phe Thr Thr Ser Thr Ser Thr Pro Val Thr Ser Asn Thr Val 620
 630 Thr Gly Glu Thr Thr Pro Phe Ser Pro Leu Val Ala Thr Ser Glu Ser Val Thr Glu Ile
 640 Thr Ala Ser Ser Phe Val Val Ser Trp Val Ser Ala Ser Asp Thr Val Ser Gly Phe 650
 660 Val Glu Tyr Glu Leu Ser Gln Glu Asp Glu Pro Gln Tyr Leu Asp Leu Pro Ser Thr
 670 Ala Thr Ser Val Asn Ile Pro Asp Leu Leu Pro Ely Arg Lys Tyr Ile Val Asn Val 680
 690 Gln Ile Ser Gln Asp Gly Glu Gln Ser Leu Ile Leu Ser Thr Ser Gln Thr Thr Ala pro
 700 Asp Ala Pro Pro Asp Pro Thr Val Asp Gln Val Asp Asp Thr Ser Ile Val Val Arg Trp 710
 720 Ser Arg Pro Gln Ala Pro Ile Thr Gly Ile Arg Ile Val Tyr Ser Pro Ser Val Glu Gln
 730 Ser Ser Thr Glu Leu Asn Leu Pro Glu Thr Ala Asn Ser Val Thr Leu Ser Asp Leu Gln
 740 FNDL 1

Fig. 5B

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Pro Gly Val Gin Tyr Asn Ile Thr Ile Tyr Ala Val Glu Glu Asn Gln Glu Ser Thr Pro 850
 Val Val Ile Gin Gin Glu Thr Thr Gly Thr Gly 870 Pro Arg Ser Asp Thr Val Pro Ser Pro Arg 880
 Asp Leu Gin Phe Val Glu Val Thr Asp Val Lys Val Thr Ile Met Trp Thr Pro Pro 890
 Ser Ala Val Thr Gly Tyr Arg Val Asp Val Ile Pro Val Asn Leu Pro Gly Glu His 910
 Gln Arg Leu Pro Ile Ser Arg Asn Thr Phe Ala Glu Val Thr Gly Leu Ser Pro Gly 930
 Val Thr Tyr Phe Lys Val Phe Ala Val Ser His Gly Arg Gln Ser Lys Pro Leu Thr Asp Ser 950
 Gln Gln Thr Thr Lys Leu Asp Ala Pro Thr Asn Leu Gln Phe Val Asn Glu Thr Asp Ser 970
 Thr Val Leu Val Arg Trp Thr Pro Pro Arg Ala Gln Ile Thr Gly Tyr Arg Leu Thr 990
 Gly Leu Thr Arg Arg Gly Gin Pro Arg Gln Tyr Asn Val Gly Pro Ser Val Ser Lys Tyr 1010
 Pro Leu Arg Asn Leu Gin Pro Ala Ser Glu Tyr Thr Val Ser Leu Val Ala Ile Lys Gly 1030
 Asn Gln Glu Ser Pro Lys Ala Thr Gly Val Phe Thr Thr Leu Gln Pro Gly Ser Ser Ile 1050
 Pro Pro Tyr Asn Thr Glu Val Thr Glu Thr Ile Val Ile Thr Trp Thr Pro Ala Pro 1070
 Arg Ile Gly Phe Lys Leu Gly Val Arg Pro Ser Gln Gly Gly Glu Ala Pro Arg Glu Val 1090
 Thr Ser Asp Ser Gly Ser Ile Val Val Ser Gly Leu Thr Pro Gly Val Glu Tyr Val Tyr 1110
 Thr Ile Gln Val Leu Arg Asp Gly Gin Gln Arg Asp Ala Pro Ile Val Asn Lys Val Val 1130
 Thr Pro Leu Ser Pro Thr Asn Leu His Leu Glu Ala Asn Pro Asp Thr Gly Val Leu 1150
 Thr Val Ser Trp Glu Arg Ser Thr Thr Pro Asp Ile Thr Gly Tyr Arg Ile Thr Thr Thr 1170
 Pro Thr Asn Gly Gin Gin Gly Asn Ser Leu Glu Val Val His Ala Asp Gln Ser Ser 1190
 Cys Thr Phe Asp Asn Leu Ser Pro Gly Leu Glu Tyr Asn Val Ser Val Tyr Thr Val Lys 1210
 Asp Asp Lys Glu Ser Val Pro Ile Ser Asp Thr Ile Ile Pro Ala Val Pro Pro Pro Thr 1230
 Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg Val Thr Trp Ala Pro Pro Pro 1250

Fig. 5C

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Ser Ile Asp Leu Thr Asn Phe Leu Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val 1280
 Ala Glu Leu Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu Pro Gly 1300
 Thr Glu Tyr Val Val Ser Ser Val Val Tyr Glu Gln His Glu Ser Thr Pro Leu Arg 1320
 Gly Arg Gln Lys Thr Gly Leu Asp Ser Pro Thr Gly Ile Asp Ser Asp Ile Thr Ala 1340
 Asn Ser Phe Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg Ile Arg 1360
 HIS HIS Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp Arg Val Pro His Ser Arg Asn 1380
 Ser Ile Thr Leu Thr Asn Lau Thr Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu 1400
 Asn Gly Arg Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp Val Pro 1420
 Arg Asp Leu Glu Val Val Ala Thr Pro Thr Ser Leu Leu Ile Ser Trp Asp Ala Pro 1440
 Ala Val Thr Val Arg Tyr Tyr Arg Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val 1460
 Gln Glu Phe Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys Pro Gly 1480
 Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg Gly Asp Ser Pro Ala Ser Ser 1500
 Lys Pro Ile Ser Ile Asn Tyr Arg Thr Gln Ile Asp Lys Pro Ser Gln Met Gln Val Val Thr 1520
 Asp Val Gln Asp Asn Ser Ile Ser Val Lys Trp Leu Pro Ser Ser Pro Val Thr Gly 1540
 Tyr Arg Val Thr Thr Pro Lys Asn Gln Pro Gly Pro Thr Lys Thr Ala Val Thr 1560
 Pro Asp Gln Thr Glu Met Thr Ile Glu Gln Pro Leu Val Gln Thr Ala Val Thr 1580
 Val Tyr Ala Gln Asn Pro Ser Gly Glu Ser Gln Pro Leu Val Gln Thr Ala Val Thr 1600
 Ile Pro Ala Pro Thr Asp Leu Lys Phe Thr Gln Val Thr Pro Thr Ser Leu Ser Ala Gln 1620
 Trp Thr Pro Pro Asn Val Gln Leu Thr Gly Tyr Arg Val Arg Val Thr Pro Lys Glu Lys 1640
 Thr Gly Pro Met Lys Glu Ile Asn Leu Ala Pro Asp Ser Ser Val Val Ser Gly 1660
 Leu Met Val Ala Thr Lys Tyr Glu Val Ser Val Tyr Ala Leu Lys Asp Thr Leu Thr Ser 1680
 FNDL 1

Fig. 5D

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Arg Pro Ala Gin Gly Val Val Thr Thr Leu Glu Asn Val Ser Pro Pro Arg Arg Ala Arg
 Val Thr Asp Ala Thr Glu Thr Thr Ile Thr Ile Ser Trp Arg Thr Lys Thr Glu Thr Ile
 Thr Gly Phe Gin Val Asp Ala Val Pro Ala Asn Gly Gin Thr Pro Ile Gin Arg Thr Ile
 Lys Pro Asp Val Arg Ser Tyr Thr Ile Thr Gly Leu Gin Pro Gly Thr Asp Tyr Lys Ile
 Tyr Leu Tyr Thr Leu Asn Asp Ala Arg Ser Ser Pro Val Val Ile Asp Ala Ser Thr
 Ala Ile Asp Ala Pro Ser Asn Leu Arg Phe Leu Ala Thr Thr Pro Asn Ser Leu Leu Val
 Ser Trp Gin Pro Pro Arg Ala Arg Ile Thr Gly Tyr Ile Ile Lys Tyr Glu Lys Pro
 Ser Pro Pro Arg Glu Val Val Pro Arg Pro Gly Val Thr Glu Ala Thr Ile Thr
 Gly Leu Glu Pro Gly Thr Glu Tyr Thr Ile Tyr Val Ile Ala Leu Lys Asn Asn Gin Lys
 Ser Glu Pro Leu Ile Gly Arg Lys Lys Thr Aso Glu Leu Pro Gin Leu Val Thr Leu Pro
 His Pro Asn Leu His Gly Pro Glu Ile Leu Asp Val Pro Ser Thr Val Gin Lys Thr Pro
 Phe Val Thr His Pro Gly Tyr Asp Thr Gly Asn Gly Ile Gin Leu Pro Gly Thr Ser
 Gin Gin Pro Ser Val Gly Gin Gin Met Ile Phe Glu Glu His Gly Phe Arg Arg Thr Thr
 Pro Pro Thr Ala Thr Pro Ile Arg His Arg Pro Arg Pro Tyr Pro Asn Val Ala
 Leu Ser Gin Thr Thr Ile Ser Trp Ala Pro Phe Gin Asn Thr Ser Glu Tyr Ile Ile Ser
 Cys His Pro Val Gly Thr Asp Glu Glu Pro Leu Gin Phe Arg Val Pro Gly Thr Ser Thr
 Ser Ala Thr Leu Thr Gly Leu Thr Arg Gly Ala Thr Tyr Asn Ile Val Glu Ala Leu
 Lys Asp Gin Gin Arg His Lys Val Arg Elu Glu Val Val Thr Val Gly Asn Ser Val Asn
 Glu Gly Leu Asn Gin Pro Thr Asp Asp Ser Cys Phe Asp Pro Tyr Thr Val Ser His Tyr
 Ala Val Gly Asp Glu Trp Glu Arg Met Ser Glu Ser Gly Phe Lys Leu Leu Cys Gin Cys
 Leu Ser Phe Gly Ser Gly His Phe Arg Cys Asp Ser Ser Arg Trp Cys His Asp Asn Gly

Fig. 5E

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Val Asn Tyr Lys Ile Gly Glu Lys Trp Asp Arg Gln Gly Glu Asn Gln Met Met Ser
Cys Thr Cys Leu Gly Asn Gly Lys Gly 2110 Phe Lys Cys Asp Pro His Glu Ala Thr Cys
Tyr Asp ASP Gly Lys Thr Tyr His Val Gly Glu Gln Trp Gln Lys Glu Tyr Leu Gly Ala
Ile Cys Ser Cys Thr Cys Phe Gly Gly 2130 Arg Gly Trp Arg Cys Asp Asn Cys Arg Arg
Pro Gly Glu Pro Ser Pro Glu Gly 2150 Thr Thr Gly Gln Ser Tyr Asn Gln Tyr Ser Gln
Arg Tyr His Gln Arg Thr Asn Thr Asn Val Asn Cys Pro Ile Glu Cys Phe Met Pro Leu
Asp Val Gln Ala Asp Arg Glu Asp Ser Arg Glu 2170 2190 2210 2230 2250 2270 2290 2310 2330 2350 2370 2390 2410 2430 2450 2470 2490 2510 2530 2550 2570 2590 2610 2630 2650 2670 2690 2710 2730 2750 2770 2790 2810 2830 2850 2870 2890 2910 2930 2950 2970 2990 3010 3030 3050 3070 3090 3110 3130 3150 3170 3190 3210 3230 3250 3270 3290 3310 3330 3350 3370 3390 3410 3430 3450 3470 3490 3510 3530 3550 3570 3590 3610 3630 3650 3670 3690 3710 3730 3750 3770 3790 3810 3830 3850 3870 3890 3910 3930 3950 3970 3990 4010 4030 4050 4070 4090 4110 4130 4150 4170 4190 4210 4230 4250 4270 4290 4310 4330 4350 4370 4390 4410 4430 4450 4470 4490 4510 4530 4550 4570 4590 4610 4630 4650 4670 4690 4710 4730 4750 4770 4790 4810 4830 4850 4870 4890 4910 4930 4950 4970 4990 5010 5030 5050 5070 5090 5110 5130 5150 5170 5190 5210 5230 5250 5270 5290 5310 5330 5350 5370 5390 5410 5430 5450 5470 5490 5510 5530 5550 5570 5590 5610 5630 5650 5670 5690 5710 5730 5750 5770 5790 5810 5830 5850 5870 5890 5910 5930 5950 5970 5990 6010 6030 6050 6070 6090 6110 6130 6150 6170 6190 6210 6230 6250 6270 6290 6310 6330 6350 6370 6390 6410 6430 6450 6470 6490 6510 6530 6550 6570 6590 6610 6630 6650 6670 6690 6710 6730 6750 6770 6790 6810 6830 6850 6870 6890 6910 6930 6950 6970 6990 7010 7030 7050 7070 7090 7110 7130 7150 7170 7190 7210 7230 7250 7270 7290 7310 7330 7350 7370 7390 7410 7430 7450 7470 7490 7510 7530 7550 7570 7590 7610 7630 7650 7670 7690 7710 7730 7750 7770 7790 7810 7830 7850 7870 7890 7910 7930 7950 7970 7990 8010 8030 8050 8070 8090 8110 8130 8150 8170 8190 8210 8230 8250 8270 8290 8310 8330 8350 8370 8390 8410 8430 8450 8470 8490 8510 8530 8550 8570 8590 8610 8630 8650 8670 8690 8710 8730 8750 8770 8790 8810 8830 8850 8870 8890 8910 8930 8950 8970 8990 9010 9030 9050 9070 9090 9110 9130 9150 9170 9190 9210 9230 9250 9270 9290 9310 9330 9350 9370 9390 9410 9430 9450 9470 9490 9510 9530 9550 9570 9590 9610 9630 9650 9670 9690 9710 9730 9750 9770 9790 9810 9830 9850 9870 9890 9910 9930 9950 9970 9990 10010 10030 10050 10070 10090 10110 10130 10150 10170 10190 10210 10230 10250 10270 10290 10310 10330 10350 10370 10390 10410 10430 10450 10470 10490 10510 10530 10550 10570 10590 10610 10630 10650 10670 10690 10710 10730 10750 10770 10790 10810 10830 10850 10870 10890 10910 10930 10950 10970 10990 11010 11030 11050 11070 11090 11110 11130 11150 11170 11190 11210 11230 11250 11270 11290 11310 11330 11350 11370 11390 11410 11430 11450 11470 11490 11510 11530 11550 11570 11590 11610 11630 11650 11670 11690 11710 11730 11750 11770 11790 11810 11830 11850 11870 11890 11910 11930 11950 11970 11990 12010 12030 12050 12070 12090 12110 12130 12150 12170 12190 12210 12230 12250 12270 12290 12310 12330 12350 12370 12390 12410 12430 12450 12470 12490 12510 12530 12550 12570 12590 12610 12630 12650 12670 12690 12710 12730 12750 12770 12790 12810 12830 12850 12870 12890 12910 12930 12950 12970 12990 13010 13030 13050 13070 13090 13110 13130 13150 13170 13190 13210 13230 13250 13270 13290 13310 13330 13350 13370 13390 13410 13430 13450 13470 13490 13510 13530 13550 13570 13590 13610 13630 13650 13670 13690 13710 13730 13750 13770 13790 13810 13830 13850 13870 13890 13910 13930 13950 13970 13990 14010 14030 14050 14070 14090 14110 14130 14150 14170 14190 14210 14230 14250 14270 14290 14310 14330 14350 14370 14390 14410 14430 14450 14470 14490 14510 14530 14550 14570 14590 14610 14630 14650 14670 14690 14710 14730 14750 14770 14790 14810 14830 14850 14870 14890 14910 14930 14950 14970 14990 15010 15030 15050 15070 15090 15110 15130 15150 15170 15190 15210 15230 15250 15270 15290 15310 15330 15350 15370 15390 15410 15430 15450 15470 15490 15510 15530 15550 15570 15590 15610 15630 15650 15670 15690 15710 15730 15750 15770 15790 15810 15830 15850 15870 15890 15910 15930 15950 15970 15990 16010 16030 16050 16070 16090 16110 16130 16150 16170 16190 16210 16230 16250 16270 16290 16310 16330 16350 16370 16390 16410 16430 16450 16470 16490 16510 16530 16550 16570 16590 16610 16630 16650 16670 16690 16710 16730 16750 16770 16790 16810 16830 16850 16870 16890 16910 16930 16950 16970 16990 17010 17030 17050 17070 17090 17110 17130 17150 17170 17190 17210 17230 17250 17270 17290 17310 17330 17350 17370 17390 17410 17430 17450 17470 17490 17510 17530 17550 17570 17590 17610 17630 17650 17670 17690 17710 17730 17750 17770 17790 17810 17830 17850 17870 17890 17910 17930 17950 17970 17990 18010 18030 18050 18070 18090 18110 18130 18150 18170 18190 18210 18230 18250 18270 18290 18310 18330 18350 18370 18390 18410 18430 18450 18470 18490 18510 18530 18550 18570 18590 18610 18630 18650 18670 18690 18710 18730 18750 18770 18790 18810 18830 18850 18870 18890 18910 18930 18950 18970 18990 19010 19030 19050 19070 19090 19110 19130 19150 19170 19190 19210 19230 19250 19270 19290 19310 19330 19350 19370 19390 19410 19430 19450 19470 19490 19510 19530 19550 19570 19590 19610 19630 19650 19670 19690 19710 19730 19750 19770 19790 19810 19830 19850 19870 19890 19910 19930 19950 19970 19990 20010 20030 20050 20070 20090 20110 20130 20150 20170 20190 20210 20230 20250 20270 20290 20310 20330 20350 20370 20390 20410 20430 20450 20470 20490 20510 20530 20550 20570 20590 20610 20630 20650 20670 20690 20710 20730 20750 20770 20790 20810 20830 20850 20870 20890 20910 20930 20950 20970 20990 21010 21030 21050 21070 21090 21110 21130 21150 21170 21190 21210 21230 21250 21270 21290 21310 21330 21350 21370 21390 21410 21430 21450 21470 21490 21510 21530 21550 21570 21590 21610 21630 21650 21670 21690 21710 21730 21750 21770 21790 21810 21830 21850 21870 21890 21910 21930 21950 21970 21990 22010 22030 22050 22070 22090 22110 22130 22150 22170 22190 22210 22230 22250 22270 22290 22310 22330 22350 22370 22390 22410 22430 22450 22470 22490 22510 22530 22550 22570 22590 22610 22630 22650 22670 22690 22710 22730 22750 22770 22790 22810 22830 22850 22870 22890 22910 22930 22950 22970 22990 23010 23030 23050 23070 23090 23110 23130 23150 23170 23190 23210 23230 23250 23270 23290 23310 23330 23350 23370 23390 23410 23430 23450 23470 23490 23510 23530 23550 23570 23590 23610 23630 23650 23670 23690 23710 23730 23750 23770 23790 23810 23830 23850 23870 23890 23910 23930 23950 23970 23990 24010 24030 24050 24070 24090 24110 24130 24150 24170 24190 24210 24230 24250 24270 24290 24310 24330 24350 24370 24390 24410 24430 24450 24470 24490 24510 24530 24550 24570 24590 24610 24630 24650 24670 24690 24710 24730 24750 24770 24790 24810 24830 24850 24870 24890 24910 24930 24950 24970 24990 25010 25030 25050 25070 25090 25110 25130 25150 25170 25190 25210 25230 25250 25270 25290 25310 25330 25350 25370 25390 25410 25430 25450 25470 25490 25510 25530 25550 25570 25590 25610 25630 25650 25670 25690 25710 25730 25750 25770 25790 25810 25830 25850 25870 25890 25910 25930 25950 25970 25990 26010 26030 26050 26070 26090 26110 26130 26150 26170 26190 26210 26230 26250 26270 26290 26310 26330 26350 26370 26390 26410 26430 26450 26470 26490 26510 26530 26550 26570 26590 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|-----------------------------------------------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GAAGAGCCTCAGAATTAACTCACTGAGACTCCGAGTCAGCCAACTCCCACCCCATCCAGTGG | | | | | | | | | | | | 2 | | | | | | | | | |
| CTTCTCGGAGTCTAAATTAGTGACTCTGAGGCTCAGTCGGTTGAGGGTGGCTAGGTCAACC | | | | | | | | | | | | | | | | | | | | | |
| e | e | p | q | n | l | i | t | e | | t | p | s | q | p | n | s | h | p | i | q | w |
| | | | | | | | | | | | | 8 | | | | | | | | | |
| | | | | | | | | | | | | 3 | | | | | | | | | |
| AATGCACACAGCCATCTCACATTTCCAAGTACATTCTCAGGTGGAGACCTAAAAAATTCTGTA | | | | | | | | | | | | | | | | | | | | | |
| TTACGTGGTGTGGTAGAGTGTAAAGGTTATGTAAGAGTCCACCTCTGGATTTTAAGACAT | | | | | | | | | | | | | | | | | | | | | |
| n | a | p | q | p | | s | h | i | s | k | y | i | l | r | w | r | p | k | n | s | v |
| | | | | | | | | | | | | 7 | | | | | | | | | |
| | | | | | | | | | | | | 4 | | | | | | | | | |
| GGCCGTTGGAAGGAAGCTACCATACCAGGCCACTTAAACTCCTACACCATCAAAGGCCTG | | | | | | | | | | | | | | | | | | | | | |
| CCGGCAACCTTCCTTCGATGGTATGGTCCGGTGAATTGAGGATGTGGTAGTTCCGGACTTAA | | | | | | | | | | | | | | | | | | | | | |
| g | r | w | k | e | a | t | i | p | g | h | l | n | s | | y | t | i | k | g | l | |
| | | | | | | | | | | | | 6 | | | | | | | | | |
| | | | | | | | | | | | | 5 | | | | | | | | | |

Figure 6 Linker 5 showing the eight constituent oligonucleotides

SUBSTITUTE SHEET

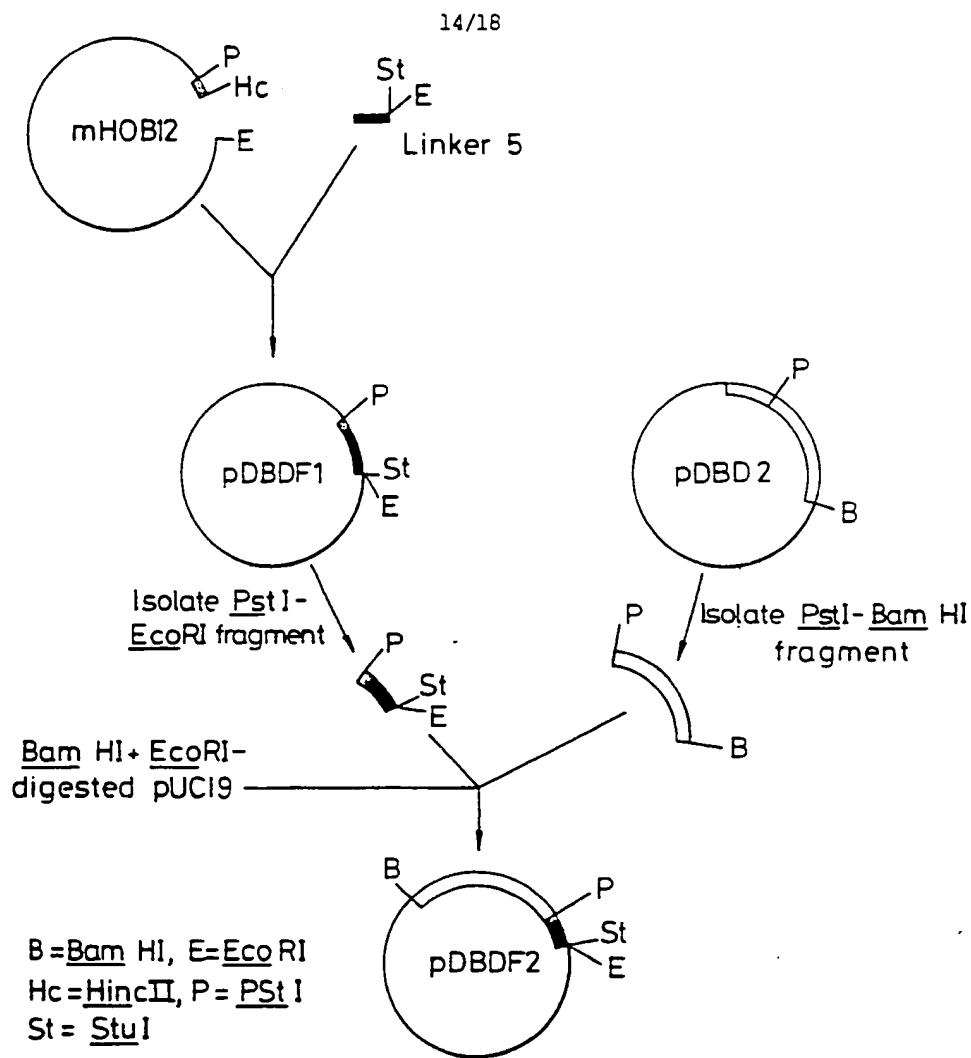


Fig. 7 Construction of pDBDF2

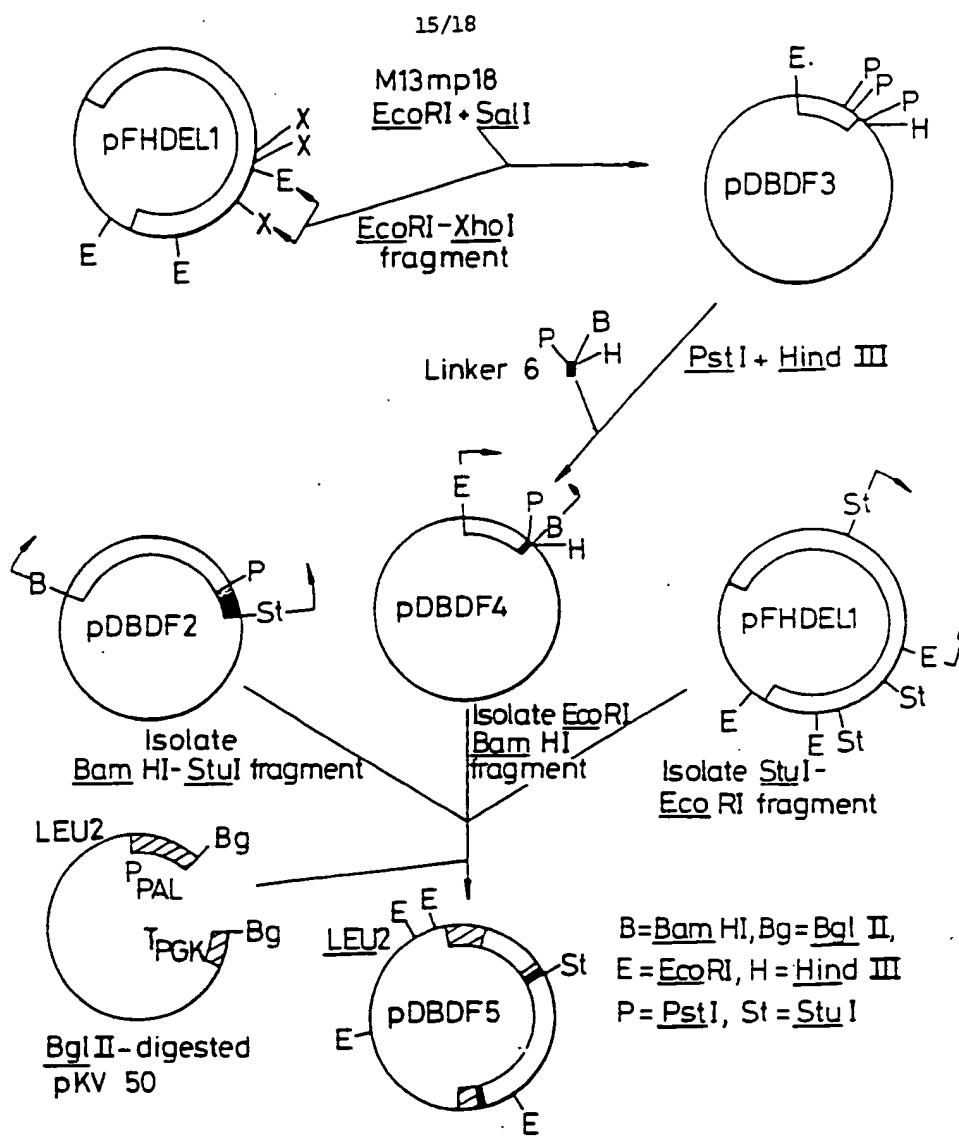


Fig. 8 Construction of pDBDF5

SUBSTITUTE SHEET

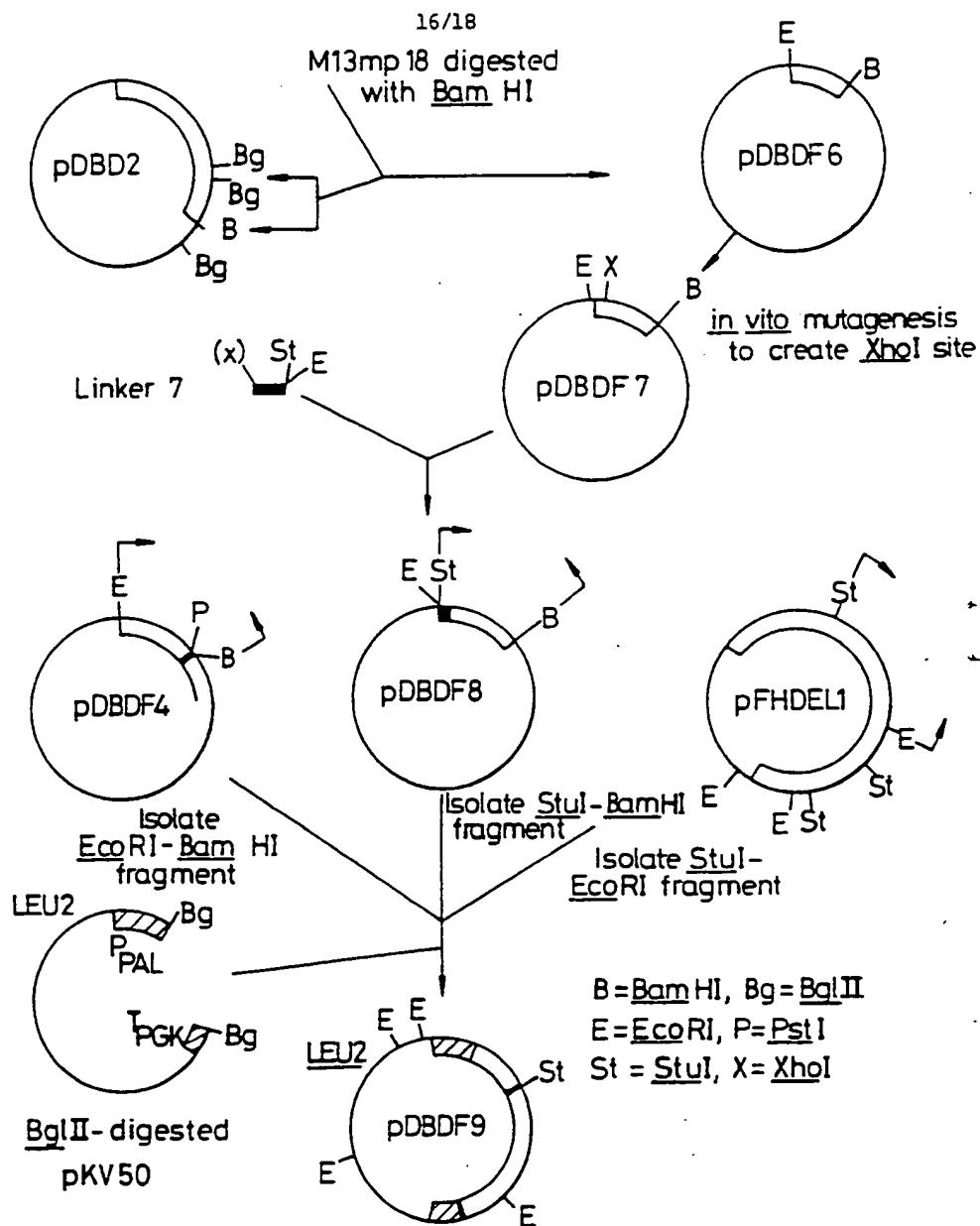


Fig. 9 Construction of pDBDF9

SUPPLEMENT SHEET

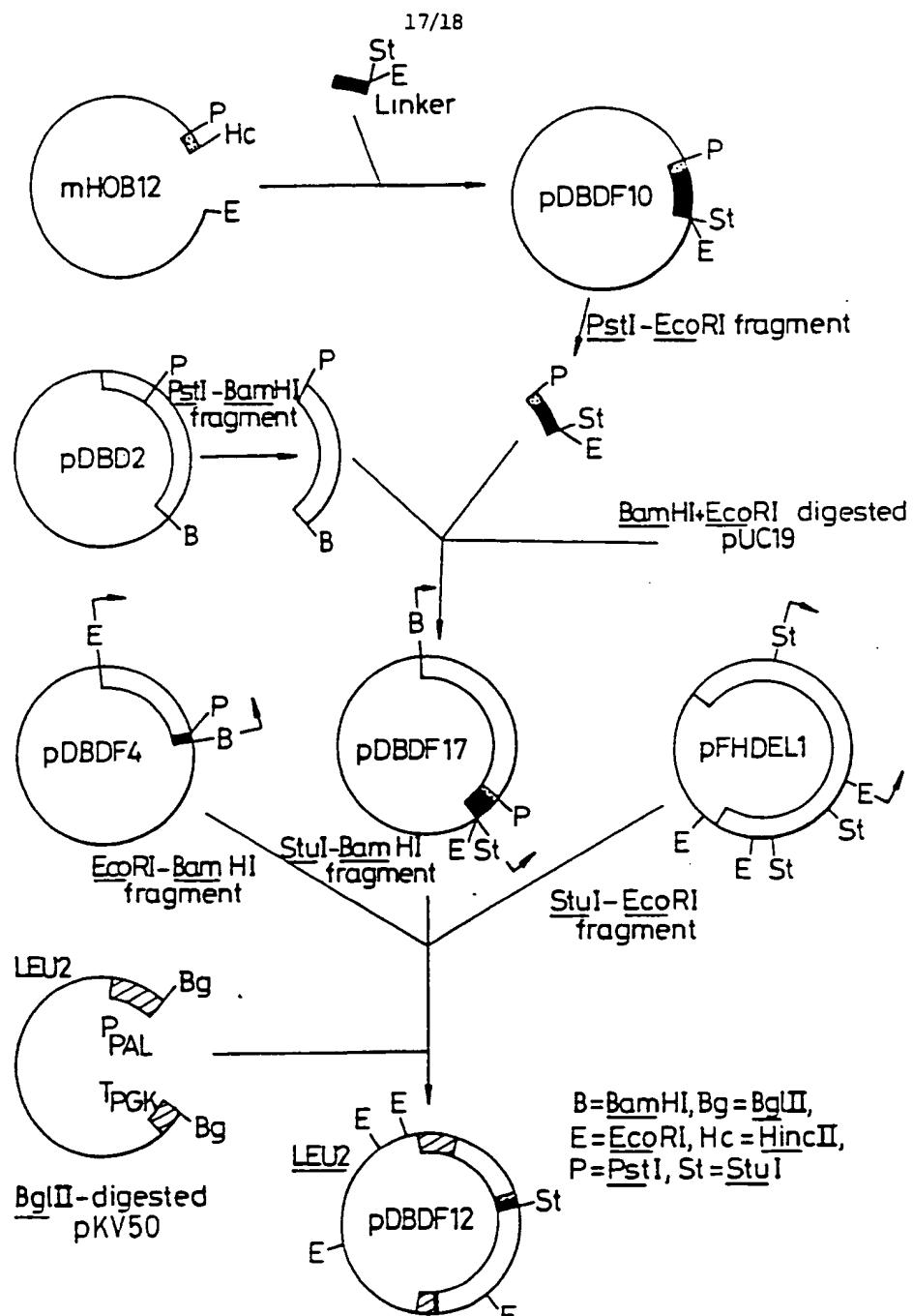
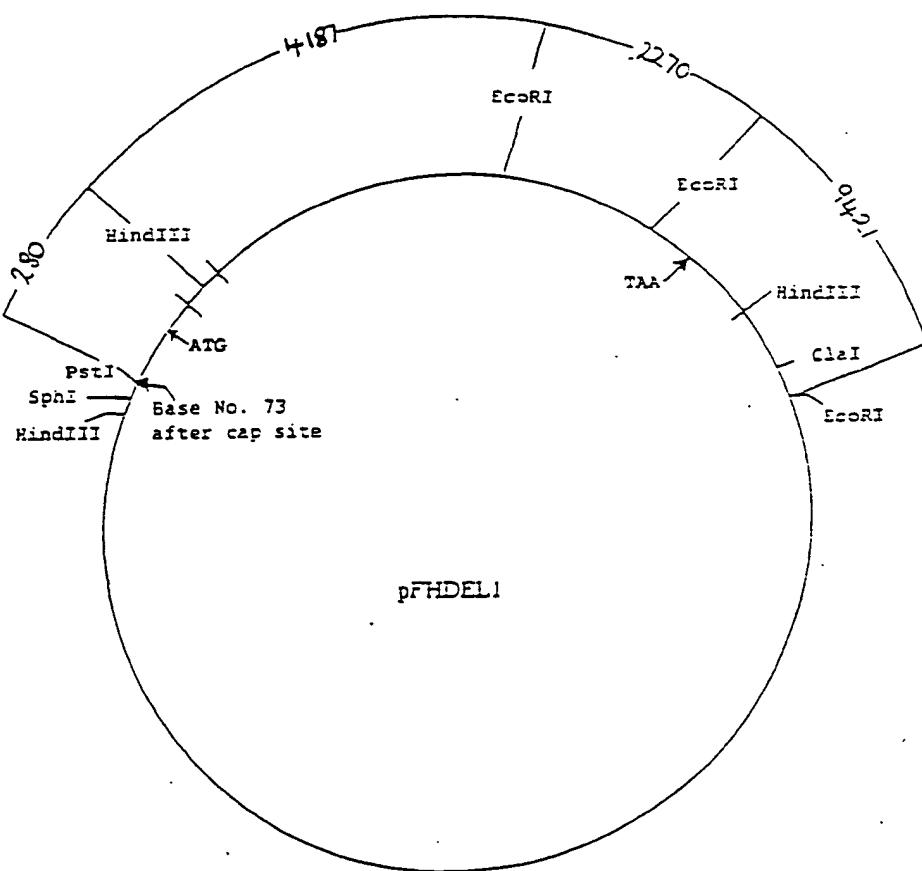


Fig. 10 Construction of pDBDF12

Figure 11

Name: pFHDEL1
Vector: pUC18 Amp^r 2860bp
Insert: hFNCcDNA - 7630bp



INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/00650

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁴

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC⁵: C 12 N 15/62, C 07 K 13/00, C 12 P 21/02

II. FIELDS SEARCHED

| Classification System ¹ | Minimum Documentation Searched ⁷ | |
|-----------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|--|
| | Classification Symbols | |
| IPC ⁵ | C 12 N, C 12 P, C 07 K | |
| Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸ | | |

III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹

| Category ¹⁰ | Citation of Document ¹¹ with indication, where appropriate, of the relevant passages ¹² | Relevant to Claim No. ¹³ |
|------------------------|---------------------------------------------------------------------------------------------------------------|-------------------------------------|
| A | EP, A, 0308381 (SKANDIGEN et al.) 22 March 1989 -- | |
| T | EP, A, 0322094 (DELTA BIOTECHNOLOGY LTD) 28 June 1989 (cited in the application) | |
| | ----- | |

* Special categories of cited documents: ¹⁰

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- "O" document relating to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"A" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

10th July 1990

Date of Mailing of this International Search Report

09.08.90

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

M. SOTELO

14
ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.

GB 9000650
SA 36670

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 31/07/90
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| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
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| | | AU-A- | 2420488 | 17-04-89 |
| | | SE-A- | 8703539 | 15-03-89 |
| | | WO-A- | 8902467 | 23-03-89 |
| EP-A- 0322094 | 28-06-89 | AU-A- | 2404688 | 18-05-89 |

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82